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Dermal uptake of nicotine from air and clothing: Experimental verification

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ABSTRACT

The current study aims to elucidate in greater detail the dermal uptake of nicotine from air or from nicotine-exposed clothes, which was demonstrated recently in a preliminary study. Six non-smoking participants were exposed to gaseous nicotine (between 236 and 304 µg/m^3) over 5 h while breathing clean air through a hood. Four of the participants wore only shorts and two wore a set of clean clothes. One week later, two of the bare-skinned participants were again exposed in the chamber, but they showered immediately after exposure instead of the following morning. The two participants who wore clean clothes on week one, were now exposed wearing a set of clothes that had been exposed to nicotine. All urine was collected for 84 h after exposure and analysed for nicotine and its metabolites cotinine and 3OH-cotinine. All participants except those wearing fresh clothes excreted substantial

amounts of biomarkers, comparable to levels expected from inhalation intake. Uptake for one participant wearing exposed clothes exceeded estimated intake via inhalation by >50%. Excretion continued during the entire urine collection period, indicating that nicotine accumulates in the skin and is released over several days. Absorbed nicotine was significantly lower after showering in one subject, but not the other. Differences in the normalized uptakes and in the excretion patterns were observed among the participants. The observed cotinine half-lives suggest that non-smokers exposed to airborne nicotine may receive a substantial fraction through the dermal pathway. Washing skin and clothes exposed to nicotine may meaningfully decrease exposure.

Keywords: *Exposure Pathway, Biomonitoring, Indoor Environment, Smoking, Skin, Metabolism*

PRACTICAL IMPLICATIONS

Dermal uptake of nicotine from the air in environments with smoking or vaping can continue for a substantial time after exposure. Wearing clean clothes substantially reduces uptake, but wearing clothes exposed to nicotine can further increase uptake. Showering shortly after exposure may reduce uptake.

1. INTRODUCTION

Exposure to nicotine via dermal contact has been widely studied, with focus on green tobacco leaves and transdermal patches.[1,2] Inhalation is typically the only pathway considered when evaluating nicotine exposure resulting from passive smoking. Dermal uptake of nicotine from air may however be an important pathway of exposure among passive smokers, including children. Recent modeling suggested that dermal uptake of certain organic compounds, including nicotine, directly from air can be a significant exposure pathway.[3,4] Weschler et al.[5] experimentally demonstrated for the first time that dermal uptake of two gas-phase phthalates, diethyl phthalate and di(n-butyl) phthalate, can be comparable to or higher than intake via inhalation. Morrison et al.[6] showed that clean clothing can

impede, while clothing that has previously absorbed/adsorbed indoor air pollutants can increase dermal uptake. We have recently demonstrated dermal uptake of airborne nicotine directly from air or from exposed clothing.[7] The air-to-skin-to-blood pathway may also be relevant with regard to thirdhand smoke, which can be associated not only with indoor surfaces, but also skin and clothes.[8] Moreover, evaluating this unexplored route of exposure is all the more important in light of the increasing adoption of relatively unregulated e-cigarettes.[9] E-cigarette use results in elevated levels of nicotine in air and on surfaces including clothing. Although the exposure conditions may differ from environments with environmental tobacco smoke (ETS), e-cigarettes are also anticipated to contribute to public secondhand exposure via the dermal pathway.[10,11]

The effect of washing/bathing on dermal uptake directly from air has not been investigated. Hand washing and showering remove skin lipids and can reduce percutaneous penetration of certain compounds.[12] Washing removed on average 96% of nicotine residue from the hands of tobacco harvesters.[13] In an *in vitro* study by Zorin et al.[14], pure nicotine and nicotine in various concentrations in water or ethanol was removed by washing three or five minutes after application on human skin. Permeation through skin continued after removing almost all nicotine from the skin surface, indicating rapid development of a nicotine reservoir in the skin itself. The cumulative concentration in the receptor compartment was however greatly reduced when the skin was rinsed after three minutes compared to five minutes. Whether washing has the potential to reduce dermal uptake of nicotine after exposure to, for example, second hand smoke is unclear.

The preliminary study of nicotine absorption from air by Bekö et al.[7] was performed on two bare-skinned and one clothed participant. Daily pooled urine samples were collected over 60 hours after a 3-hour exposure in a climate chamber, where nicotine was dosed by continuous smoking of cigarettes using a smoking machine. The subsequent excretion of nicotine and its metabolites indicated that skin acts as a reservoir after exposure to airborne nicotine. We concluded that ionization of nicotine on

the surface of skin or within the stratum corneum does not substantially impede uptake. The study was, however, limited in extent and detail. Furthermore, the exposures occurred in a setting with very high particle levels, which confounded interpretation of the results. The current study aims to expand our knowledge on the dermal uptake of nicotine from air and clothing, by conducting experiments on a larger number of participants, with more controlled concentrations using pure gas-phase nicotine, a longer period of exposure and collection of individual urine sample over a longer time. Additionally, it assesses the effect of showering immediately after exposure on the dermal uptake of nicotine.

2. METHODS

2.1 Human participants and experimental plan

A total of six male participants were exposed to nicotine to study dermal uptake to bare skin as well as investigate the impact of showering and clothing. Figure 1 presents a diagram showing the overall experimental design. One experiment investigated the dermal uptake of nicotine directly from air. Four healthy males between 50-68 years of age (P1-P4) participated. They were exposed to air containing nicotine at elevated concentration. The exposure period was five hours. The participants wore only shorts and breathed clean air through a breathing hood.[5] The participants were asked to shower the night before and again in the morning of the day following exposure. One week later two of the participants (P3 and P4) were again exposed in the chamber, but they showered immediately after exposure. In a companion experiment, during the first week two participants (P5 and P6; age 36 and 50 years, respectively) wore a set of clean clothes (underpants, socks, shirt, pants and gloves) comprised of cotton, polyester and rayon while being exposed to nicotine in a fashion identical to the bare-skinned participants. During the second week, these participants were exposed wearing identical shirt, socks and gloves that had been exposed to nicotine at an air concentration of ~500 µg/m^3 for 16 days, then ~250 µg/m^3 for 11 days. They wore full-length pants and underpants that had been cleaned and *not* exposed. All participants were non-smokers and were not exposed to environmental tobacco

smoke (ETS) or other sources of nicotine during the days prior to the exposure and during the subsequent urine collection period.

2.2 Nicotine in chamber air

Nicotine (Table S1) in aqueous solution (1%) was dosed using a step-motor driven syringe in a 55 m^3 climate chamber ventilated at an air exchange rate of 0.7 h^{-1} (the average air temperature during exposure was 29.8 °C in week 1 and 27.6 °C in week 2). Droplets of the solution were delivered onto a heated stainless steel plate (35 °C), which evaporated the nicotine into the air. The dosing rate was 1.88 mL/h. To minimize sorption of nicotine on the chamber surfaces, the walls, floor and ceiling of the chamber were covered with thin polyethylene sheet. Dosing began two days prior to exposure, in order to establish steady-state nicotine concentration in the chamber air.

Nicotine in the chamber air was determined by collecting 5 to 6 L of air (100-150 mL/min) on Tenax TA filled stainless-steel tubes. One sample was taken every hour during exposure and seven duplicate samples were collected as well. The tubes were analyzed via thermal desorption gas chromatography/mass spectrometry (TD-GC/MS) according to ISO 16000-6.[15] Field blanks from each day of exposure were also analyzed and the nicotine concentration was in all cases below the limit of detection (< 1 µg/m³) (see Supporting Information for calibration data; Figures S1 and S2). Triplicate air samples (5 L of air at a flow 40 mL/min) were collected from one of the breathing hoods under conditions similar to when the participants were wearing hoods. The average measured nicotine concentration in breathing hoods was 3.7 µg/m^3 (st.dev. = 0.58), less than 2% of the average nicotine concentration in the chamber air during these experiments. Figure S3 shows an image of the exposure chamber, nicotine dosing and air sampling.

2.3 Urine collection and analyses

One to two urine samples were collected immediately before the participants entered the chamber. All urine was collected for 84 hours after entering the chamber. For participants P1, P2 and P5 post-exposure urine samples were pooled; one pooled sample contained urine collected within the first 12 hours after the beginning of exposure, the second, third and fourth pooled samples contained urine collected during the subsequent three 24-hour periods. For participants P3, P4 and P6 all individual urine samples were collected, weighed and analyzed in order to study in greater detail the impact of clothing and showering immediately after exposure. Pooled samples were also prepared for these participants; they were reconstituted from the individual samples and analyzed together with the pooled samples of participants P1, P2 and P5 (Figure S4). Urine samples were analyzed for nicotine and two of its metabolites, cotinine and 3-hydroxycotinine (including their conjugates after enzymatic hydrolyses) via LC-MS with isotope dilution quantification, as described in Bekö et al.[7]. The limits of quantification (LOQ) for nicotine, cotinine and 3-hydroxy-cotinine were 0.10, 0.05 and 0.12 µg/L, respectively.

2.4 Data analyses

The mass of nicotine and each metabolite excreted was determined by multiplying the absolute concentration (µg/L) in the pooled samples by the corresponding urine volume (L). For each pooled sample, the amounts of nicotine and its metabolites were corrected by the corresponding amounts measured in the pre-samples collected before entering the chamber, scaled by the ratio of the pooled sample volume to the pre-sample volume. This allows us to obtain an estimate of dose resulting from the 5 hours in the chamber. This correction for background exposure is somewhat conservative, as the background urinary concentrations of nicotine and the two metabolites were somewhat higher in week 2 compared to week 1 (nicotine 0.18 vs. 0.05 (1/2 LOD) µg/L, cotinine 1.73 vs. 0.28 µg/L and 3OH-cotinine 3.89 vs. 0.51 µg/L, respectively, for participants P3-P6 who were exposed both weeks). For participants P3, P4 and P6, whose individual samples were analyzed, the excretion mass rate for

each interval was calculated by dividing the excreted mass per urination by the elapsed time since the previous urination.

The half-lives of nicotine and its metabolites were determined from the mass excreted in the last two pooled samples for each participant. For participants P3, P4 and P6, half-lives were also calculated from a regression of the last 48 hours of excretion rates (appropriately log-transformed). Half-lives that were greater than two-times the population mean or less than zero were excluded.

The total uptake of nicotine was calculated from the excreted amounts of nicotine and its metabolites using the following molecular weights (g/mol): nicotine: 162, cotinine: 176, 3-hydroxy-cotinine: 192. We estimated the amount of nicotine absorbed by assuming that 90% of nicotine and its metabolites are excreted via urine and that the three metabolites and their conjugates constitute 85% of metabolites excreted in urine.[16] We then subtracted the amount of nicotine inhaled from hood air (IU), calculated by the following equation:

$$IU = BR*C_{air}*f*t = 9\ \mu g \qquad (1)$$

where BR is the breathing rate (0.7 m^3/h), C_{air} is the average air concentration in the hood (3.7 µg/m^3), f is the fraction of inhaled nicotine that is absorbed (0.7; see Bekö et al.[7] for details) and t is the exposure time (5h).

Finally, total uptakes of nicotine were normalized first by the chamber air concentrations of nicotine and then by the participant's exposed body surface area (BSA, based on the method of DuBois and DuBois[17]). Ninety percent of BSA was used for bare-skinned participants and for participants wearing fresh clothes. For participants wearing exposed clothes, we assumed that their normalized exposure from air during the 5 hours in the chamber will be the same as when they were wearing fresh clothes. This fraction of their uptake was normalized by 90% of BSA. The remaining fraction of the uptake was attributable to the exposed shirt, gloves and socks and was normalized by 52% of BSA.[18] The

final normalized uptake of participants wearing exposed clothes was thus determined using the following equation:

$$Uptake = \frac{\frac{M_2}{C_2} - \frac{M_1}{C_1}}{52\%\ BSA} + \frac{\frac{M_1}{C_1}}{90\%BSA} \qquad (2)$$

where M_1 and M_2 are the background and hood concentration corrected absorbed dose while wearing fresh clothes and absorbed clothes, respectively (µg) and C_1 and C_2 are the corresponding nicotine air concentrations during the two exposure periods (µg/m^3).

The research protocol was approved by the Capital Region of Denmark Committee for Research Ethics (case no. H-16018670).

3. RESULTS

The physiological parameters of the six participants and the nicotine air concentrations are summarized in Table 1. The average nicotine concentration in chamber air was between 236 µg/m^3 and 240 µg/m^3 in the first week of the experiment and between 281 µg/m^3 and 304 µg/m^3 in the second week (Figure S5).

3.1 Excreted amounts of nicotine and metabolites

Following exposure, the concentrations of nicotine and nicotine metabolites in the urine of the bare-skinned participants (P1-P4) quickly increased considerably above the levels measured before they entered the chamber. They excreted a significant amount of nicotine and nicotine metabolites (Table 1 and Figures 2 and S6). Substantial differences were observed in the net excretion patterns among participants. Participant P1 excreted large amounts of nicotine and cotinine, while participant P2 excreted much more 3OH-cotinine than nicotine or cotinine, reflecting faster metabolism by participant P2. Participant P3 excreted similar amounts of the three compounds the first exposure week. In week two, when he showered immediately after exposure, he excreted substantially smaller amounts of nicotine and cotinine, but not 3OH-cotinine. Participant P4, however, excreted twice as

much nicotine, slightly more cotinine and comparable amount of 3OH-cotinine in week 2 compared with week 1, when showering did not occur immediately after exposure. Differences were also seen between the two clothed participants (Figures 3 and S7). Participant P5 excreted similar amounts of cotinine and 3OH-cotinine and less nicotine, while participant P6 excreted substantially more 3OH-cotinine than nicotine and cotinine, both when wearing clean clothes and exposed clothes.

The excreted amounts of nicotine and the two metabolites obtained from pooled samples were compared with those from individual samples for participants P3, P4 and P6 (Figures 3 and S6, and Table S2). The identical trends and similar absolute values obtained by the two methods indicate that the results from reconstituted pooled samples reliably represent the observed exposure and can be analyzed together with the data from participants P1, P2 and P5, who collected pooled urine only.

Net excretions of the three compounds continued to increase throughout the 84 h post-exposure period for all participants. Nicotine absorption associated with the chamber exposure was not completely captured even after 3.5 days of urine collection. This is supported by the excretion rates shown in Figure 4, especially in the case of the nicotine metabolites that exhibit delayed excretion and longer elimination half-lives compared to nicotine. Excretion rates peaked 1-1.5 days after exposure began (somewhat later for metabolites) and then decayed. Half-lives of the three compounds are shown in Table 2 and Figure S8. The average half-lives for nicotine, cotinine and 3OH-cotinine were 28h (SD14), 35h (SD 15), and 34h (SD 19), respectively. For participants P3, P4 and P6, half-lives of cotinine based on total mass excreted on consecutive days (24h pools) were generally consistent with those based on a regression of excretion rates from individual samples.

3.2 Nicotine uptake

The back-calculated amount of dermally absorbed nicotine (dose) varied among the participants (Table 1). The average dose was 650 µg for the bare-skinned participants during week 1 (range 460-

820 µg). After normalization by body surface area and chamber air concentration, the average dose was 1.53 µg/m^2/(µg/m^3) (range 1.22-1.8; Figure 5). For participants who showered immediately after exposure, normalized absorbed nicotine was lower than without showering, by 52% for participant P3 and 6% for participant P4. For the two participants wearing clean clothes, the amount of absorbed nicotine was 25 µg and 85 µg (0.06 and 0.18 µg/m^2/(µg/m^3)), substantially lower than for the bare-skinned participants. It increased to 470 µg and 1144 µg (1.6 and 3.1 µg/m^2/(µg/m^3)) while wearing clothes (not pants) previously exposed to nicotine. The clothing was responsible for ~95% of this uptake (Figure 5).

4. DISCUSSION

4.1 Urine concentrations and absorbed dose

Peak concentrations in the 12- or 24-hour pooled urine samples of the bare-skinned participants (between 10 and 85 ng/ml, data not shown) were similar to those of the two bare-skinned participants in Bekö et al.[7] and comparable to levels measured among non-smokers in hospitality environments before the smoking ban. Peak concentration in the individual urine samples were slightly higher (nicotine: 102 ng/ml (P3), 89 ng/ml (P4); cotinine: 71 ng/ml (P3), 38 ng/ml (P4); 3OH-cotinine: 123 ng/ml (P3), 68 ng/ml (P4)), approaching levels measured in light smokers.[19] The total absorbed dose of nicotine for the bare-skinned participants in week 1 (average 650 µg) was similar to the minimum uptake estimated for bare-skinned participants based on 60h excretions in Bekö et al.[7] (570 µg). Given the long elimination half-lives and the observation that metabolites are still being excreted at the end of urine collection, these doses underestimate the total nicotine absorbed.

The airborne nicotine concentration was higher in the earlier study (420 µg/m^3), where the source of nicotine was environmental tobacco smoke. However, exposure lasted longer in the current study (5 h vs. 3 h). Moreover, the absence of particles in the current study is expected to increase the fraction of total nicotine in the gas-phase.[20] Nicotine air concentrations were higher than concentrations

reported for most environments where smoking occurs. They were comparable to the levels reported for smoking sections of UK and German pubs and to mean levels measured in German discotheques.[21,22] The slightly higher average concentration in the second week reflects a lower rate of nicotine removal by participants; only two participants were seated in the chamber compared to three exposed participants in the first week.

Nicotine and metabolite concentrations measured in pooled samples for the participants wearing clean clothes were low (15 ng/ml). Clean clothes are expected to be protective for compounds like nicotine that meaningfully sorb to clothing fibers, reducing the rate of transport to the skin.[23] When the participants wore a set of exposed clothes, the concentrations were comparable or higher than for the bare-skinned participants (peak nicotine: 29 ng/ml (P5), 55 ng/ml (P6); peak cotinine: 46 ng/ml (P5), 43 ng/ml (P6); peak 3OH-cotinine: 48 ng/ml (P5), 148 ng/ml (P6)). These concentrations are higher than observed in Bekö et al.[7], which is probably due to a longer pre-exposure of the clothes, a longer wearing time in the chamber and a larger body surface area covered with exposed clothes. The total uptakes of participants P5 and P6 with exposed clothes covering only part of the body (~50%) were similar or higher than uptakes of the bare-skinned (~90% exposed) participants, indicating a higher uptake rate when wearing exposed clothes.[6] Compared to the earlier nicotine study, the clothing in the current experiment had been exposed to elevated concentrations of nicotine for a longer time, and had likely come much closer to equilibrium with nicotine in the chamber air.

4.2 Accumulation in skin and cotinine half-lives

Excretion of nicotine and its metabolites (above generally observable background levels) continued throughout the entire period of urine collection. This observation supports earlier conclusions that skin acts as a reservoir for chemicals that accumulated during exposure and delivers them into the blood after exposure.[7,24,25] Comparison of background nicotine and metabolite concentrations measured in the pre-exposure samples collected in weeks 1 and 2 further supports this hypothesis.

The average concentrations of nicotine, cotinine and 3OH-cotinine in the pre-exposure samples in week 1 were <LOD, 0.28 and 0.51 µg/L, respectively. During week 2 they were 0.18, 1.73 and 3.89 µg/l, respectively. None of the individual background levels in week 1 was higher than the corresponding value from week 2. The slightly higher pre-exposure levels in week 2 may be due to somewhat higher background exposures during the days prior to entering the chambers in week 2 (for which we have no indication). More likely, the higher starting concentrations reflect metabolism and excretion of residual nicotine present in the body one week after the first exposure. Additionally, the ratio of week 2 to week 1 background urine concentrations were higher for the bare-skinned participants (range 1-18) than for the clothed participants (1-5), possibly due to the much lower exposure of the latter participants during week 1.

The observed cotinine half-lives are similar to, but somewhat larger than those of non-smokers exposed to ETS (Table 2 and Figure S8). Smokers take in most of their nicotine by inhalation and have cotinine half-lives of ~16 h.[26] Exposure to nicotine in airborne ETS results in a much longer half-life (27h).[26] The average dermal-only half-life, observed in the current study is 35 h based on pooled samples and 33h based on individual samples (P3, P4 and P6). These values suggest that exposure of nonsmokers to nicotine in airborne ETS is from a combination of inhalation and dermal absorption, since the resulting half-life is between that for mainstream smoking and dermal absorption. However, given the small number of participants, coupled with the variability of the measured half-lives, these results should be interpreted with caution.

4.3 Comparison with inhalation uptake

We can estimate what the inhalation uptake during the 5h chamber exposure would be, had the subjects not been wearing a breathing hood. Using a breathing rate of 0.7 m^3/h [18], the measured nicotine air concentrations, and a value of 0.7 for the fraction of inhaled nicotine absorbed,[7] the inhalation uptake is between 580 and 750 µg, depending on the nicotine concentration in the air on

311 the day of exposure. These doses are comparable to the observed dermal uptakes of the bare-skinned
312 participants in week 1 (average 650 µg, Table 1). However, the 5 h exposure time is too short for
313 dermal uptake from the gas-phase to reach steady state.[27] Longer exposure time would result in
314 dermal uptake rates closer to steady-state values and larger than uptake via inhalation. Wearing
315 previously exposed clothes can further increase dermal absorption. The uptake of nicotine for
316 participant P6 was 50% higher than the corresponding inhalation uptake without a hood would have
317 been even though only about half of the participant's skin was covered by nicotine-exposed clothes.
318
319 *4.4 Differences in normalized uptake*
320 Differences were observed in the normalized uptakes between the four bare-skinned participants in
321 week 1 as well as between the two participants wearing fresh or exposed clothes. Contrary to the
322 results of our earlier studies indicating increasing dermal uptake with age for lipophilic
323 compounds,[5,24] nicotine uptake during week 1 was the lowest for the oldest participant. However, in
324 our previous study the older of the two participants (identical to P4 in the current study) had a higher
325 normalized uptake compared with his 32 years younger counterpart.[7] The older of the two clothed
326 participants in the current study had higher normalized uptake both with fresh and exposed clothes.
327 Age therefore cannot explain the differences in uptake between the participants. The differences could
328 have been caused to a certain extent by differences in skin type (thickness, hydration, pH, buffering
329 capacity), sweating, desquamation, lipid content and other skin conditions such as that related to
330 filaggrin gene loss-of-function mutation.[28,29] The type of clothes worn after exiting the exposure
331 chamber may have had an effect as well. The substantial difference between the uptakes of the two
332 participants wearing exposed clothes may have been additionally influenced by the cloth-skin gap
333 (i.e., the clothes fitting more tightly on the participant with larger BSA (2.24 m^2 vs. 1.93 m^2)).[24,23]
334 Other parameters, such as geometry and permeability of the fabric, laundering and exposure of the
335 clothes to nicotine prior to wearing were identical for the two participants. Studies with more

participants are warranted to better elucidate the effect of age and clothing on dermal uptake of nicotine.

Both participants P3 and P4 had a lower normalized uptake in week 2 when they showered immediately after exposure in the chamber. After exiting the exposure chamber in week 1, the participants donned clothing, which is anticipated to reduce absorption due to transfer of nicotine from skin lipids to clothing.[27,23] Showering in week 2 likely removed more effectively a fraction of the nicotine in skin surface lipids that had not yet penetrated into the epidermis and the dermis. The reduction of uptake after showering was much smaller in case of the older participant. It is plausible that nicotine was absorbed more quickly from the surface of the skin, as older skin tends to be drier (less ionization) and has a thinner epidermis.[30,31] Additionally, we did not control for the duration of showering, water temperature and soap applied. These factors influence skin dryness, skin pH and consequently nicotine ionization and removal.

4.5 Factors affecting nicotine clearance

The differences among the excretion patterns of the six individuals were substantial. For example, participant P2 metabolized nicotine fast and excreted more than 80% of the total (nicotine + metabolites expressed as nicotine equivalents) in the form of metabolites (~50% as 3OH-cotinine), while participant P1 excreted 55% as metabolites (~15% as 3OH-cotinine). Given the small number of participants, we cannot reach clear conclusions regarding differences in nicotine metabolism following dermal uptake. Nonetheless, some discussion of factors that influence nicotine clearance seems appropriate.

The availability and activity of the enzymes responsible for nicotine and cotinine metabolism may partially explain the observed differences.[32] Variations in urine flow and urine pH, may also influence the results. It is noteworthy that there were different excretion patterns for the same individual – in

week 1 participant P4 rapidly metabolized nicotine, excreting only 22% of the total excreted amount as nicotine; in week 2, his metabolism of nicotine was slower (36% excreted as nicotine); in the previous study, the same individual (participant 1 in Bekö et al.[7]) metabolized nicotine even more slowly (44% excreted as nicotine). Nicotine is primarily metabolized in the liver, indicating that it depends on liver blood flow and therefore on physiological factors such as diet and exercise.[16] Although animal studies suggest potential metabolism to a small extent in other organs, comparable human studies are lacking. We cannot evaluate the contribution from metabolism in the skin, as is known to occur for other compounds (e.g., DEHP).[33]

Diurnal rhythms have been shown to affect nicotine clearance. We observed peak nicotine excretion rates to occur at or just before midnight (Figure S9). This is most apparent in the results of participant P4, who urinated more frequently than the other participants. Hepatic blood flow falls and nicotine clearance decreases during sleep. Gries et al.[34] modeled nicotine clearance in an experiment of 48-hour constant intravenous nicotine bitartrate administration to 11 subjects for 48 hours. In contrast to the results shown in Figure S9, the earlier investigators found that nicotine clearance peaked around 11 AM and was lowest between 6 PM and 3 AM. The difference may reflect exposure via dermal absorption in the present study versus an intravenous pathway in the cited study. Gries et al.[34] also found that eating a meal increased clearance on average by 42% at peak, which occurred one hour after beginning the meal. The effect of the meal lasted nearly three hours. Taken together, circadian rhythms and changes in food ingestion and urine flow may explain the diurnal excretion rates of nicotine and the fluctuating ratios of individual excreted amounts of the three compounds (Figure S10).

For the reasons discussed above, it is unclear whether age plays a role in the observed differences in metabolism. Participants P1 and P2, with different metabolic patterns, were close in age. The older clothed participant (P6) metabolized cotinine very fast and excreted nearly 60% of the total excreted

amount of nicotine equivalent as 3OH-cotinine both weeks of the experiment, compared to 35-50% in case of the younger participant P5. However, the oldest participant P4 metabolized nicotine relatively quickly in week 1, although he metabolized it substantially slower in our earlier study. Gourlay and Benowitz[35] did not find differences in steady-state nicotine plasma or estimated plasma clearance in three age groups with nicotine patches. However, decreased clearance of nicotine has been reported for subjects above 65 years compared to adults between 22 and 43 years,[36] perhaps reflecting reduced liver blood flow.[37] Cotinine clearance is much slower and more dependent on enzyme activity, which does not change with age.[38] Indeed, the range for 3OH-cotinine/cotinine ratios among the participants was substantially smaller than that of the cotinine/nicotine or 3OH-cotinine/nicotine ratios (Figure S10).

5. CONCLUSIONS

Following our pilot study,[7] this more extensive study supports our earlier finding that nicotine can be dermally absorbed directly from air at rates comparable to or higher than via inhalation. Wearing clean clothes significantly decreases short-term uptake, while wearing exposed clothes increases uptake. Similar to contact exposure, nicotine absorbed dermally from air or clothing accumulates in the skin and is released over a period of several days, perhaps up to a week. The cotinine half-life observed in the present study, compared to cotinine's reported half-life following ETS exposure, suggests that a fraction of the exposure of non-smokers to ETS may occur through dermal absorption. Uptake and metabolism of nicotine after dermal exposure via air varies substantially between individuals. In addition to skin condition, genetic variations in metabolic enzymes, age and diet may be responsible for the variation. Washing the skin after exposure may decrease the amount of absorbed nicotine. The efficacy of skin washing likely depends on a number of factors and warrants further investigation. Frequent laundering of clothes that are regularly exposed to tobacco smoke or nicotine from vaping is anticipated to reduce nicotine uptake through skin.

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Tables and Figures

Table 1. Nicotine air concentrations during exposure, net amount of nicotine and the two metabolites excreted over 84 hours after entering the chamber (corrected for background concentrations before entering the chamber), absorbed nicotine dose (corrected for background concentration and nicotine concentration in the breathing hood), and normalized uptake determined from the pooled urine samples.

Participant	Age	BSA (m^2)	Date of exposure	Aver. nicotine conc. in air ± SD ($\mu g/m^3$)	Excreted Nicotine (µg)*	Excreted Cotinine (µg)*	Excreted 3OH-Cotinine (µg)*	Estimated Dose (µg)*	Uptake normalized by adjusted BSA** & air conc. ($\mu g/m^2/\mu g/m^3$)
P1	50	2.16	27.9.2016	236±22	279	275	124	823	1.80
P2	51	2.07	28.9.2016	240±23	82.9	183	270	616	1.38
P3	55	1.92	27.9.2016	236±22	183	193	225	711	1.74
P3-shower			5.10.2016	281±19	90.7	91.4	172	409	0.84
P4	68	1.73	28.9.2016	240±23	78.1	129	189	457	1.22
P4-shower			4.10.2016	304±26	152	148	160	543	1.15
P5-fresh clothes	36	1.93	28.9.2016	240±23	2.4	11.2	15.4	25	0.06
P5-exposed clothes			5.10.2016	281±19	106	149	146	470	1.62
P6-fresh clothes	50	2.24	27.9.2016	236±22	14.7	18.0	48.1	85	0.18
P6-exposed clothes			4.10.2016	304±26	192	215	584	1144	3.10

* background corrected (see section 2.4)

** 90% BSA was used for bare-skinned participants and for participants wearing fresh clothes. For participants wearing exposed clothes, equation (2) was applied to normalize by adjusted BSA.

Table 2. Half-lives (h) of nicotine, cotinine and 3OH-cotinine based on last two consecutive 24-h excretion rates (pooled samples) and successive-urination excretion rates (individual samples; P3, P4 and P6 only).

Participant	**Nicotine**	**Cotinine**	**3OH-cotinine**
P1	34	*	*
P2	54	25	26
P3	9 (12)	19 (23)	43 (*)
P3-shower	37 (25)	27 (40)	17 (22)
P4	38 (33)	33 (39)	17 (22)
P4-shower	24 (23)	43 (34)	38 (40)
P5-fresh clothes	21	42	55
P5-exposed clothes	25	51	72
P6-fresh clothes	* (*)	17 (20)	17 (19)
P6-exposed clothes	11 (12)	62 (42)	22 (23)
Average; SD	28; 14 (21; 9)	35; 15 (33; 9)	34; 19 (25; 8)

* negative or unrealistically large

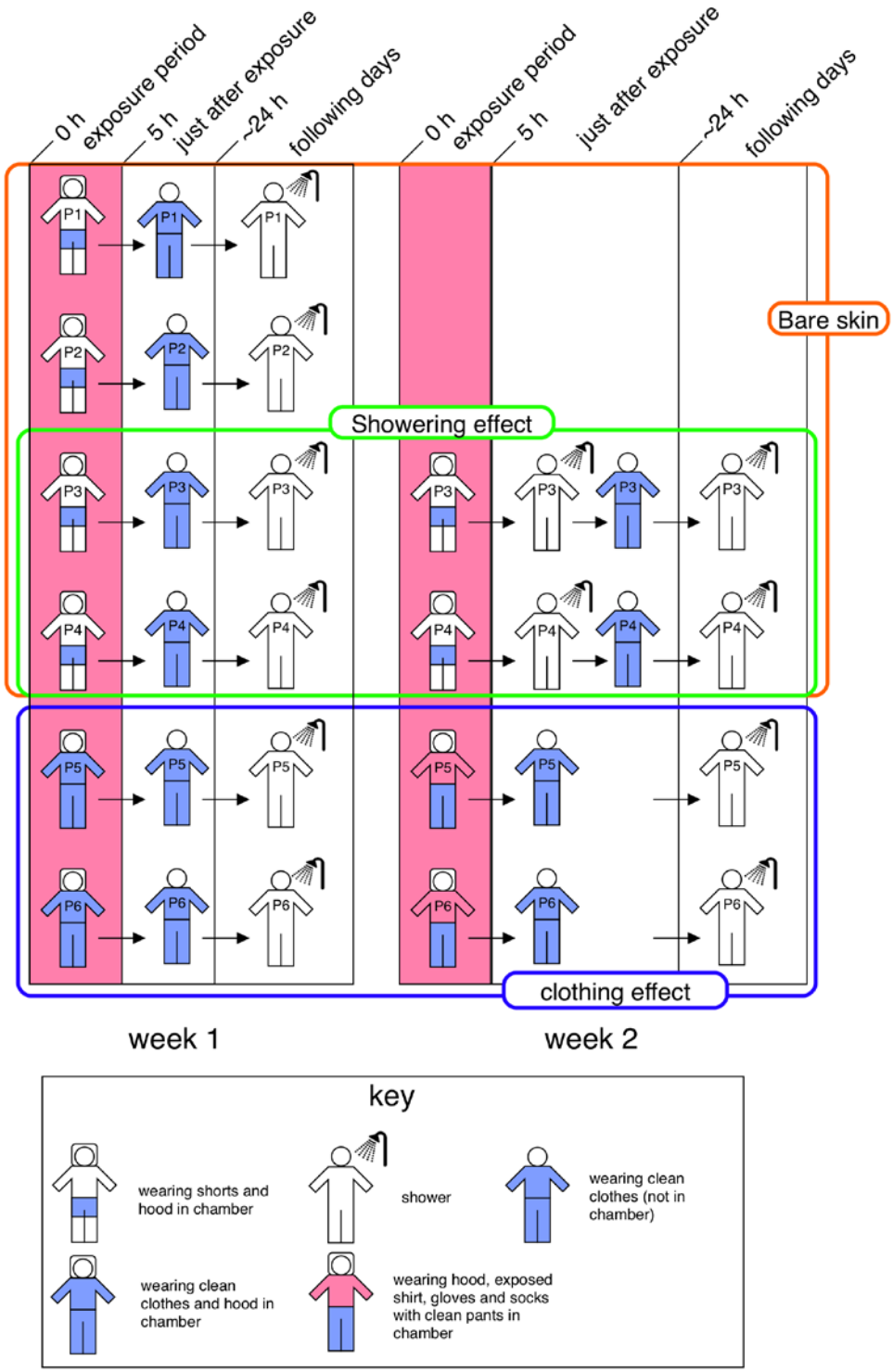


Figure 1. Experimental plan

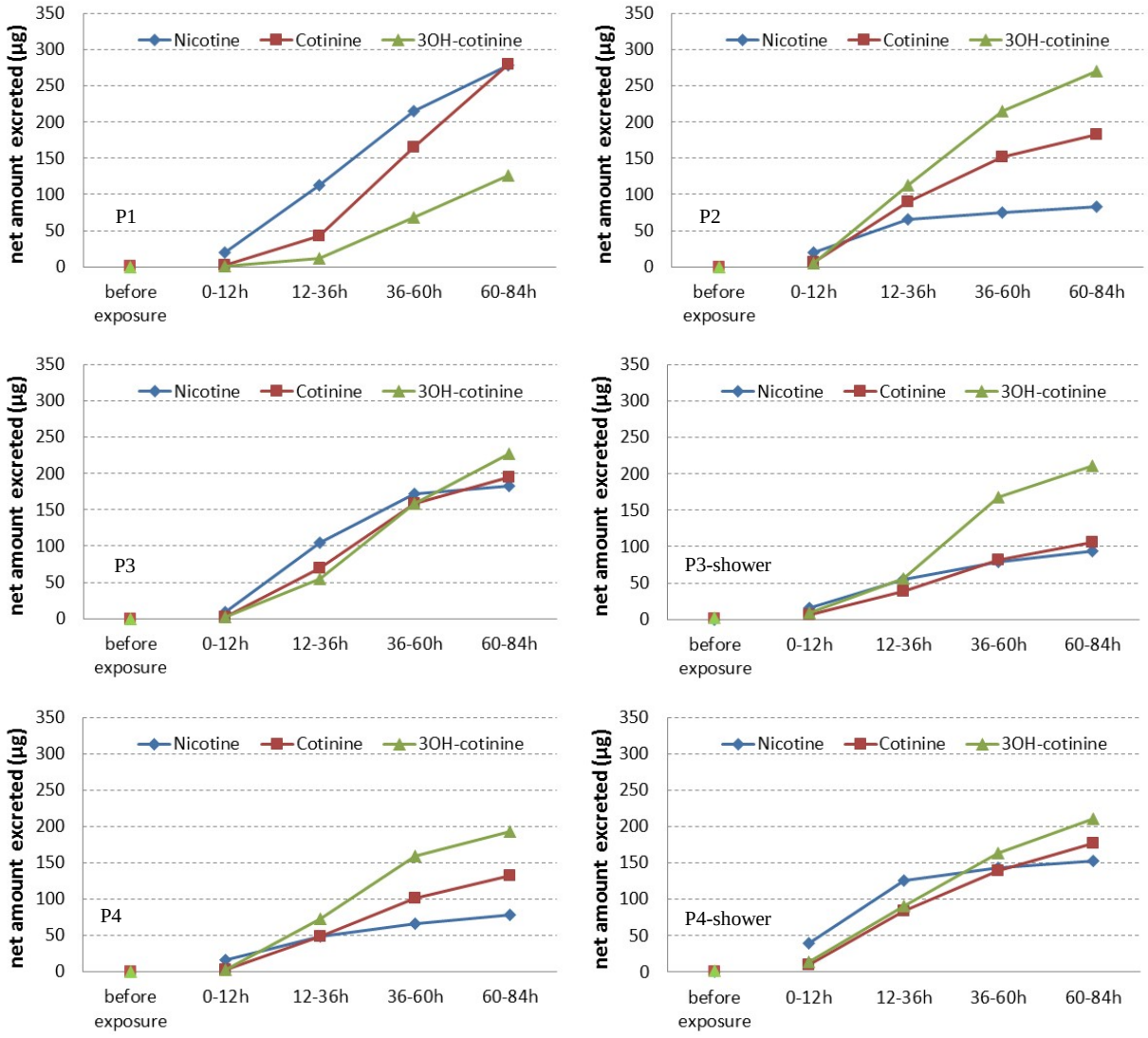


Figure 2. Net amount of excreted nicotine and the two metabolites for the four bare-skinned participants (P1-P4). Participants P3 and P4 showered immediately after exposure on the second week (right), but not the first (left).

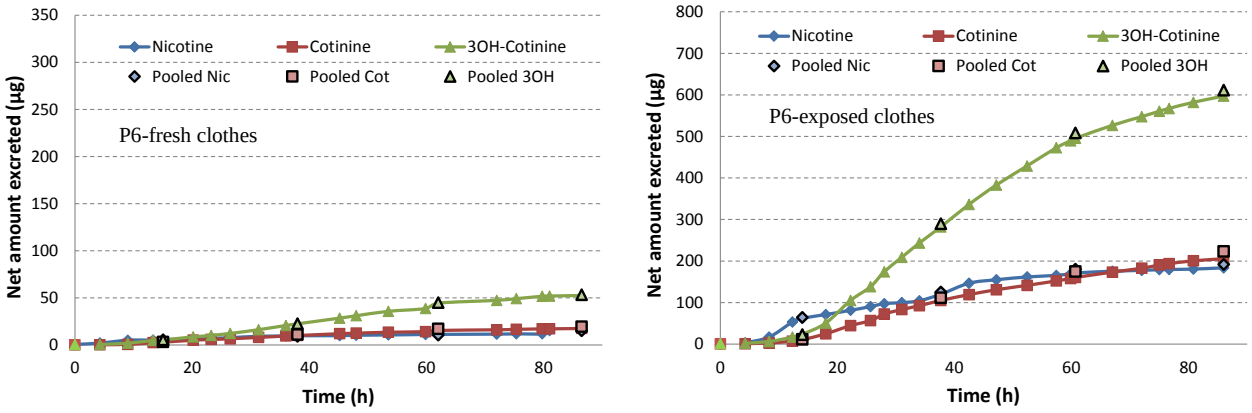
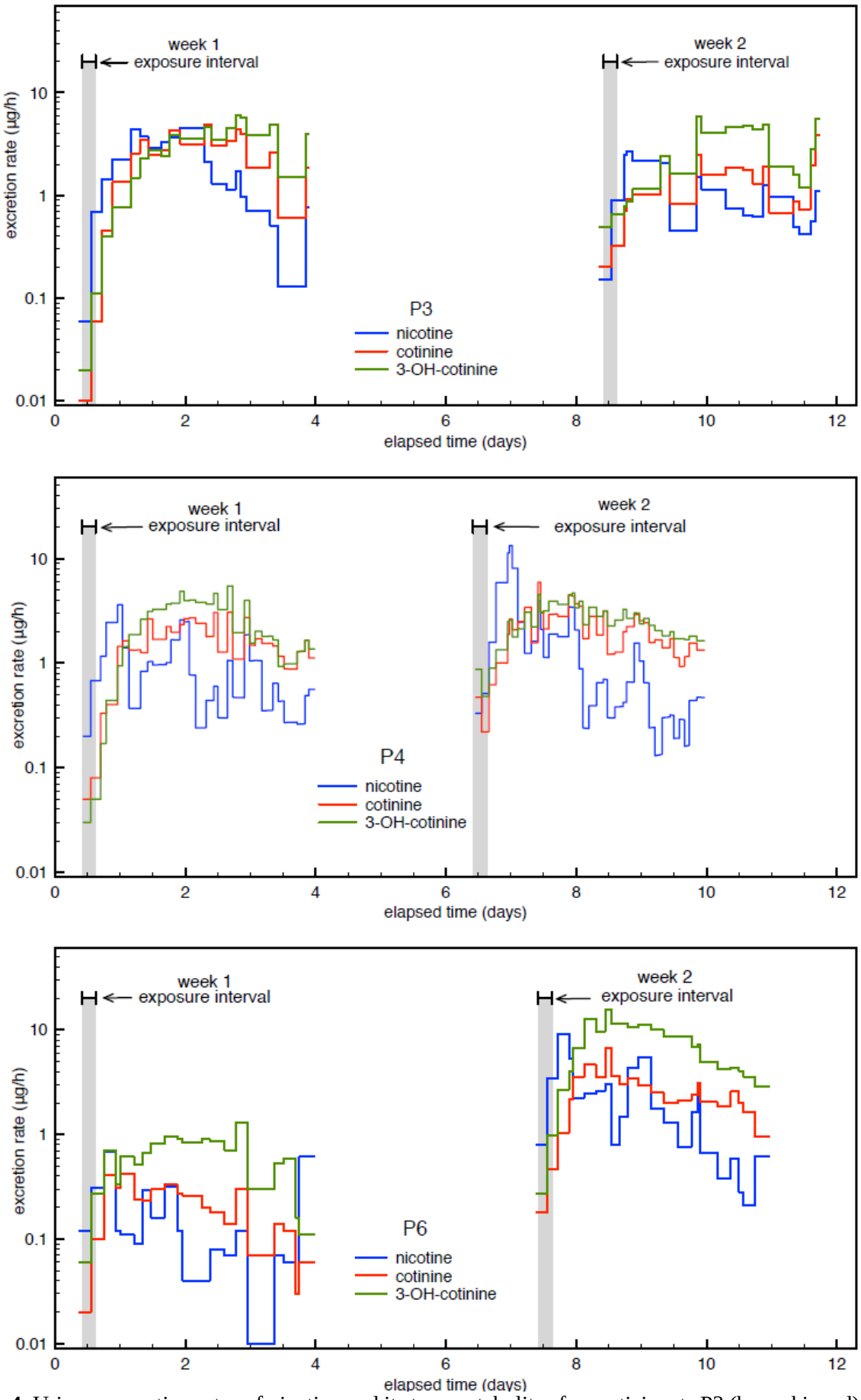
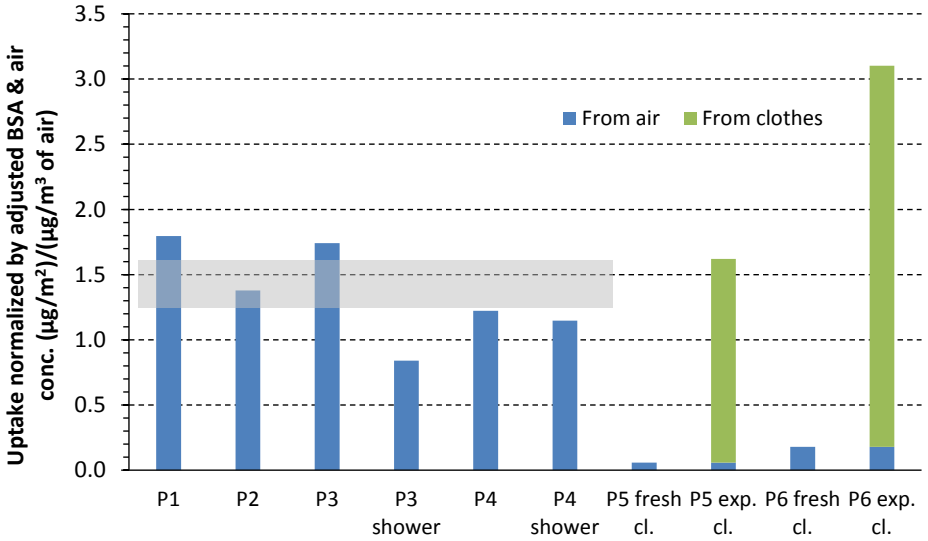


Figure 3. Net amount of excreted nicotine and the two metabolites for one of the clothed participants, P6. Data from both the individual and pooled urine samples are shown for comparison. Note the different scales on the vertical axis. (See the Supporting Information for this comparison for the other participants.)



552
553 **Figure 4**. Urinary excretion rates of nicotine and its two metabolites for participants P3 (bare-skinned), P4
554 (bare-skinned) and P6 (clothed).



555
556 **Figure 5**. Dermally absorbed nicotine normalized by chamber air concentration and adjusted body surface
557 area. The grey horizontal bar indicates the range of inhalation intake for participants P1-P4, normalized by air
558 concentration and corresponding BSA (90% of total BSA).
559